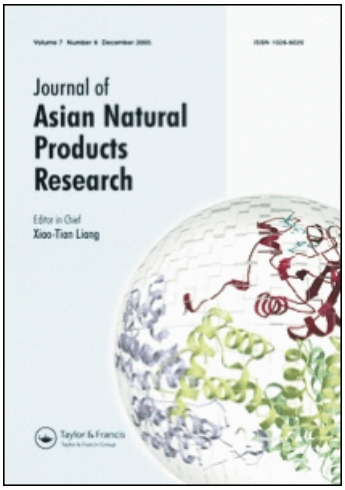

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Two new cardenolides from the roots of *Streptocaulon griffithii*

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Two new cardenolides, 3β,5β,14β-trihydroxyl-card-16,20(22)-dienolide (**1**) and 3-*O*-β-D-glucopyranosyl-5β,14β-dihydroxyl-card-16,20(22)-dienolide (**2**), together with seven known compounds identified as digitogenin (**3**), 16-*O*-acetylgitoxigenin (**4**), periplogenin (**5**), 16-*O*-acetylperiplogenin (**6**), periplogenin digitoxoside (**7**), periplogenin-3-*O*-β-D-glucopyranoside (**8**) and periplogenin-3-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-digitoxopyranoside (**9**) were isolated from the roots of *Streptocaulon griffithii*. Their structures were elucidated on the basis of spectroscopic data.

Keywords: *Streptocaulon griffithii*; Asclepiadaceae; Cardenolides; 3β,5β,14β-Trihydroxyl-card-16,20(22)-dienolide; 3-*O*-β-D-Glucopyranosyl-5β,14β-dihydroxyl-card-16,20(22)-dienolide

1. Introduction

Streptocaulon griffithii belongs to the Asclepiadaceae family and is mainly distributed in the southwestern region of China. As a folk medicine plant of the Dai minority, its roots are traditionally used for the treatment of dysentery, influenza and gastricism [1]. Some cardenolides showing strong antiproliferative activity have been isolated from *S. juventas* in this genus [2], but there was no report on the chemical constituents of *S. griffithii.* As a result, we have undertaken a systematic investigation of the chemical constituents of this plant. This paper reports the isolation and structure elucidation of two new cardenolides, 3β,5β,14β-trihydroxyl-card-16,20(22)-dienolide (**1**) and 3-*O*-β-D-glucopyranosyl-5β,14β-dihydroxyl-card-16,20(22)-dienolide (**2**) (figure 1), together with seven known compounds (**3**)–(**9**) isolated from *S. griffithii*.

2. Results and discussion

Compound **1** was obtained as a white powder, mp 277–278°C (MeOH), $[\alpha]_D^{20}$ − 6.25 (*c* 0.02, MeOH), and showed positive reactions with Legal and Liebermann–Burchard reagents. The molecular formula of **1** was determined to be $C_{23}H_{32}O_5$ by HREIMS with *m*/*z* 388.2224

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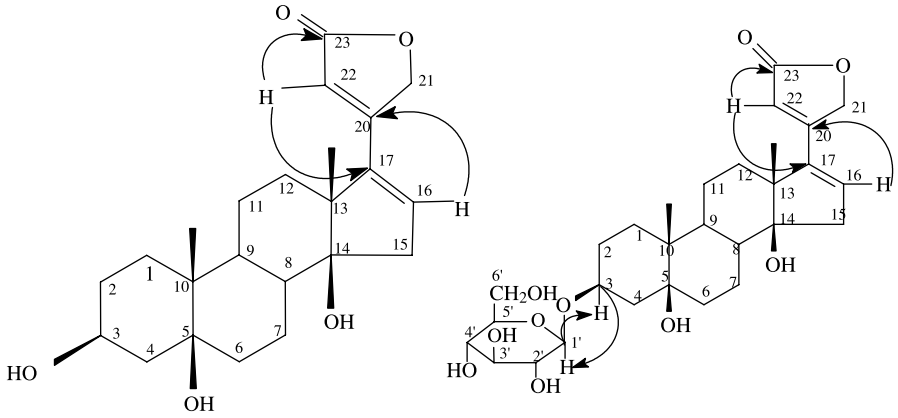

Figure 1. The structures and key HMBC correlations of compounds **1** and **2**.

$[M]^+$. Its UV spectrum showed two absorption bands at 221 and 270 nm, suggesting the presence of an α,β-unsaturated γ-lactone of cardenolides and other more unsaturated moieties. Its IR spectrum showed absorption bands at 3438 (OH), 1782, 1745 (C═O) and 1618 (C═C) cm^{-1}, indicating the presence of a carbonyl group and an alkenyl group in the molecule.

The ^{1}H-NMR spectrum of **1** showed a pair of methylene protons at δ 4.98 and 5.08 (1H each, dd, $J = 16.8$, 1.8 Hz, H-21a and H-21b), an olefinic proton at δ 5.94 (1H, br s, H-22), and two methyl protons at δ 0.95 and 1.25 (3H each s, H_3-18, H_3-19), respectively. In addition, a signal at δ 4.11 (1H, m, H-3) representing one oxygenated proton at C-3 as well as another signal at δ 6.19 (1H, dd, $J = 3.0$ Hz), indicating the presence of an additional olefinic proton, were also observed in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum of **1** displayed 23 characteristic signals of cardenolides, including a carbonyl group (δ 175.2, C-23) and two methyl groups (δ 15.1 and 15.6, C-18 and C-19), together with two pairs of olefinic carbons (δ 110.2, 132.6, 142.9 and 159.7), and three oxygenated carbons (δ 66.9, 74.0 and 84.6). The ^{13}C-NMR data of **1** (table 1) were similar to those of periplogenin (**5**) [3], except that the signals at C-16 and C-17 for **5** were replaced by a pair of olefinic carbon signals (δ 132.6 and 142.9) for **1**, accompanied by the signals at C-15, C-20 and C-22 for **5** being markedly shifted. These results indicate that there is one additional double bond located at C-16 and C-17 in **1**. This was further supported by its ^{1}H–^{1}H COSY, HMQC and HMBC spectra. In the

Table 1. ^{13}C-NMR data for compounds **1** and **2** (150 MHz, δ in ppm, in CD_3OD).

Position	***1***	***2***	*Position*	***1***	***2***	*Position*	***1***	***2***
1	24.1	26.5	11	22.5	24.7	21	71.5	73.5
2	26.6	26.9	12	37.6	39.3	22	110.2	112.0
3	66.9	78.3	13	51.2	53.1	23	175.2	177.2
4	35.7	36.0	14	84.6	86.6	Glc-1′		101.9
5	74.0	75.1	15	39.2	41.0	2′		75.9
6	33.6	34.6	16	132.6	134.8	3′		78.2
7	19.5	21.4	17	142.9	144.9	4′		71.6
8	39.2	41.1	18	15.1	16.8	5′		78.1
9	39.8	40.8	19	15.6	17.4	6′		62.7
10	38.9	41.7	20	159.7	161.9			

HMBC spectrum of **1** (figure 1), correlations could be observed between H-16 (δ 6.19) and C-20 (δ 159.7), H-22 (δ 5.94) and C-17 (δ 142.9), and H-22 (δ 5.94) and C-23 (δ 175.2), which demonstrated the location of •[16,17]. This characteristic structure was confirmed by the UV data. Thus **1** was determined to be 3β,5β,14β-trihydroxyl-card-16,20(22)-dienolide.

Compound **2** was obtained as a yellow powder, mp 172–174°C (MeOH), $[\alpha]_D^{20}$ + 15.9 (*c* 0.05, MeOH), and showed positive reactions with Legal and Liebermann–Burchard reagents. Its molecular formula was established to be $C_{29}H_{42}O_{10}$ by HRFABMS with *m/z* 573.2657 $[M + Na]^+$. The UV spectrum showed two absorption bands at 221 and 273 nm. The EIMS data of **2** were the same as for **1** at *m/z* 388, 370, 352 and 316, which indicated the presence of the same aglycone. On acid hydrolysis by co-HPTLC, **2** afforded the same aglycone spot as **1**, together with glucose using authentic samples [4]. The ^{13}C- and ^{1}H-NMR spectra of **2** were similar to those for **1** (table 1), except for a marked downfield shift of the carbon signal at C-3 (78.3, δ 11.3 downfield shifted) in the ^{13}C-NMR spectrum, together with sequenced signals for glucose at δ 101.9, 75.9, 78.2, 71.6, 78.1 and 62.7 (table 1). The glucosyl group could be deduced to be in the β configuration from its coupling constant for the anomeric proton signals at δ 4.40 (1H, d, J = 7.8 Hz, H-1′). Correlations between H-3 (δ 4.21) and C-1′ (δ 101.9) and H-1′ (δ 4.40) and C-3 (δ 78.3) could clearly be observed in the HMBC spectrum of **2** (figure 1). Consequently, the structure of **2** was determined to be 3-*O*-β-D-glucopyranosyl-5β,14β-dihydroxyl-card-16,20(22)-dienolide (figure 1).

Compounds **1** and **2** are new natural constituents possessing the characteristic skeleton of 16,20(22),23-α,β,γ-unsaturated δ-lactone. In addition, other cardenolides ((**3**)–(**9**)) were also isolated and identified [digitogenin [6] (**3**), 16-*O*-acetyldigitoxigenin [7] (**4**), periplogenin [3] (**5**), 16-*O*-acetylperiplogenin [3] (**6**), periplogenin digitoxoside [8] (**7**), periplogenin-3-*O*-β-D-glucopyranoside [9] (**8**) and periplogenin-3-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-digitoxopyranoside [4] (**9**)] by comparison of their ^{1}H- and ^{13}C-NMR spectral data with reported data.

3. Experimental

3.1 General experimental procedures

Melting points were determined using a Fisher Johns apparatus and are uncorrected. UV spectra were recorded with a Philips PYE Unican Pu8800 spectrophotometer. IR spectra were obtained using KBr disks and a Perkin-Elmer 983G spectrophotometer. NMR spectra were recorded on an INOVA 600 spectrometer. EIMS and FABMS were recorded using a Micromass ZabSpec spectrometer. TLC employed precoated silica gel plates (5–7 μm) and silica gel for column chromatography (H, 200–300 mesh) was from Qingdao Haiyang Chemical Group.

3.2 Plant material

The roots of *Streptocaulon griffithii* Hook.f. were collected from Yunnan province, China, in 2001, and identified by Professor Zai-Lin Li of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, China, where a voucher sample has been deposited.

3.3 Extraction and isolation

The dried radixes (13 kg) of the plant material were successively extracted with 95% EtOH and 50% EtOH (each 65 l × 3) under reflux. The resultant extract was combined and dried under reduced pressure to give a concentrated extract (1500 g). The latter was subsequently suspended in water and partitioned successively with petroleum ether, $CHCl_3$, EtOAc and n-butanol. The $CHCl_3$ fraction (300 g) was subjected to column chromatography on silica gel (100–200 mesh), and gradient eluted with $CHCl_3$:MeOH (100:0–40:60) to give 25 fractions. Fractions 7–11 were combined and purified on MPLC (silica gel H, 5 × 20 cm) eluted with $CHCl_3$:MeOH. Subsequently, further purification on Sephadex LH-20 columns was carried out to give compounds **1** (10 mg), **3** (50 mg), **4** (12 mg), **5** (9 mg) and **6** (13 mg). The n-butanol fraction (98 g) was chromatographed on a MPLC (silica gel H, 10 × 20 cm) column eluted with $CHCl_3$:MeOH:H_2O (90:9:1) to give 12 fractions. Fractions 4–7 were combined and further purified on a ODS column. The latter was gradient eluted with MeOH:H_2O (6:4–8:2) to give compounds **2** (13 mg), **7** (20 mg), **8** (22 mg) and **9** (11 mg).

3.3.1 Compound 1 (3β,5β,14β-trihydroxyl-card-16,20(22)-dienolide). White powder. $[\alpha]_D^{20}$ − 6.25 (*c* 0.02, MeOH); mp 277–278°C (MeOH). Showed positive reactions with Legal and Liebermann–Burchard reagents. UV (MeOH) λ_{max} (log ε) (nm): 221 (5.74 × 10^3), 270 (9.62 × 10^3); IR (KBr) ν_{max} (cm^{-1}): 438 (OH), 1782, 1745 (C═O) and 1618 (C═C); ^{1}H-NMR (CD_3OD, 600 MHz): δ 0.95 and 1.25 (3H each, s, H-18, 19), 4.11 (1H, m, H-3), 4.98 and 5.08 (each 1H, dd, J = 16.8, 1.8 Hz, H_2-21), 5.94 (1H, br s, H-22), 6.19 (1H, d, J = 3.0 Hz, H-16); ^{13}C-NMR (CD_3OD, 150 MHz) see table 1; EIMS *m/z* 388 $[M]^+$(4), 370 (5), 352 (10), 316 (100); HREIMS *m/z* 388.2224 $[M]^+$ (calcd for $C_{23}H_{32}O_5$, 388.2250).

3.3.2 Compound 2 (3-*O*-β-D-glucopyranosyl-5β,14β-dihydroxyl-card-16,20(22)-dienolide). Yellow powder. $[\alpha]_D^{20}$ + 15.94 (c 0.05, MeOH); mp 172–174°C (MeOH). Showed positive reactions with Legal and Molish reagents. UV (MeOH) λ_{max} (log ε) (nm): 221 (1.08 × 10^4), 273 (1.32 × 10^4); IR (KBr) ν_{max} (cm^{-1}): 3414 (OH), 1744, 1730 (C═O) and 1618 (C═C); ^{1}H-NMR (CD_3OD, 600 MHz): δ 0.95 and 1.25 (each 3H, s, H-18, 19), 3.17 (1H, dd, J = 7.8, 9.6 Hz, H-2′), 3.27 (1H, m, H-5′), 3.30 (1H, m, H-4′), 3.36 (1H, t, J = 9.0 Hz, H-3′), 3.64, 3.85 (each 1H, m, H-6′), 4.21 (1H, m, H-3), 4.40 (1H, d, J = 7.8 Hz, anomeric-H), 5.00 and 5.12 (each 1H, dd, J = 16.8, 1.8 Hz, H_2-21), 5.98 (1H, br s, H-22); ^{13}C-NMR (CD_3OD, 150 MHz) see table 1; EIMS *m/z* 388 (5), 370 (10), 352 (65), 316 (100); HRFABMS *m/z* 573.2657 $[M + Na]^+$ (calcd for $C_{29}H_{42}O_{14}$ + Na, 573.2675).

3.3.3 Compound 3 (digitogenin). White needles. $[\alpha]_D^{20}$ + 4.14 (*c* 0.07, MeOH); mp 229–232°C. Showed positive reactions with Legal and Liebermann–Burchard reagents. All the spectral data were completely consistent with digitogenin [6].

3.3.4 Compound 4 (16-*O*-acetylgitoxigenin). Yellow amorphous powder (MeOH). $[\alpha]_D^{20}$ − 8.89 (*c* 0.045, MeOHl); mp 225–228°C. All the spectral data were completely consistent with 16-*O*-acetylgitoxigenin [7].

3.3.5 Compound 5 (periplogenin). White needles. $[\alpha]_D^{20} + 16.67$ (*c* 0.04, MeOH); mp 238°C. Showed positive reactions with Legal and Liebermann–Burchard reagents. All the spectral data were completely consistent with periplogenin [3].

3.3.6 Compound 6 (16-*O*-acetylperiplogenin). Yellow amorphous powder. $[\alpha]_D^{20} - 9.64$ (*c* 0.05, MeOH); mp 142–144°C. Showed positive reactions with Legal and Liebermann–Burchard reagents. All the spectral data were completely consistent with 16-*O*-acetylperiplogenin [3,6].

3.3.7 Compound 7 (periplogenin digitoxoside). White needles. $[\alpha]_D^{20} + 8.82$ (*c* 0.03, MeOH); mp 145–146°C. Showed positive reactions with Legal and Molish reagents. All the spectral data were completely consistent with periplogenin digitoxoside [8].

3.3.8 Compound 8 (periplogenin-3-*O*-β-D-glucopyranoside). White amorphous powder. $[\alpha]_D^{20} - 20.45$ (*c* 0.04, MeOH); mp 145–146°C. Showed positive reactions with Legal and Molish reagents. All the spectral data were completely consistent with periplogenin-3-*O*-β-D-glucopyranoside [9].

3.3.9 Compound 9 (periplogenin-3-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-digitoxopyranoside). White amorphous powder. $[\alpha]_D^{20} + 2.00$(*c* 0.05, MeOH); mp 145–146°C. Showed positive reactions with Legal and Molish reagents. All the spectral data were completely consistent with periplogenin-3-*O*- β-D-glucopyranosyl-(1 → 4)-*O*-β-D-digitoxopyranoside [4].

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